

## Effect of dietary lipids on renal function in rats with subtotal nephrectomy

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**Effect of dietary lipids on renal function in rats with subtotal nephrectomy.** Female rats with 1-3/4 nephrectomy were divided in two groups and pair fed for five weeks diets differing in their linoleic acid content. Five weeks after subtotal nephrectomy, values for glomerular filtration rate and renal plasma flow were significantly higher and the values of blood pressure significantly lower in rats fed a diet rich in linoleic acid. Systolic blood pressure averaged  $156 \pm 5.6$  mm Hg in high and  $215 \pm 8.1$  mm Hg in low linoleic acid-fed rats. Differences in the values of blood pressure between the two groups were observed three weeks after subtotal renal ablation and persisted throughout the period of observation. Inulin clearance averaged  $0.89 \pm 0.07$  ml/min in the high and  $0.44 \pm 0.05$  ml/min in the low linoleic acid group. Protein excretion in the urine was significantly less in rats fed the high linoleic acid diet ( $36.9 \pm 4.4$  mg/24 hr) than in those fed the low linoleic acid diet ( $90.1 \pm 12.5$  mg/24 hr). The weight of the remnant kidney five weeks after subtotal renal ablation was greater in rats fed a low linoleic acid diet as compared to those fed a high linoleic acid diet ( $P < 0.05$ ). Glomerular lesions were more severe in rats fed a low linoleic acid diet than in those fed a high linoleic acid diet. Feeding high linoleic acid diets to normal and subtotally nephrectomized rats increased the content of linoleic and arachidonic acid in renal cortex and medulla. It is concluded that a diet with a high linoleic acid content improves renal function and prevents marked increases in blood pressure in rats with partial nephrectomy. The mechanisms responsible for this effect remain to be determined.

The role of dietary modification on the progression of experimental renal disease has generated renewed interest in recent years [1, 2]. Most of the studies have dealt with the effects of protein and/or phosphorus content of the diet on the progression of renal disease in animals with experimentally-induced renal injury. Few reports, however, have addressed the influence of dietary lipid composition on the progression of renal disease in animals with experimental renal failure, particularly after subtotal renal ablation.

Feeding a diet high in linoleic acid (HLAD) to rats with subtotal renal ablation has been reported to result in lower serum creatinine levels and less proteinuria than that observed in similar rats fed a low linoleic acid diet (LLAD) [3]. No differences were observed in blood pressure levels of the two groups. An enhanced renal production of arachidonic acid metabolites via the cyclooxygenase pathway (prostaglandins),

with a wide spectrum of physiological activities was suggested as one of the potential mechanisms responsible for these effects, since linoleic acid is an ubiquitous dietary precursor of arachidonic acid in tissues. Hirschberg et al [4], on the other hand, found no differences in the levels of serum creatinine or urea, and degree of proteinuria in rats with subtotal renal ablation fed diets with varying concentrations of polyunsaturated fatty acids (PUFA). It has also been demonstrated that the degree of salt-induced hypertension is greater in normal rats fed a diet low in linoleic acid for four weeks [5]. Scharschmidt, McGarry and Berger have reported recently that feeding a diet rich in eicosapentaenoic acid, a precursor of relatively inactive prostanoid metabolites which compete with those derived from arachidonic acid, to rats with subtotal renal ablation resulted in lower creatinine clearances and higher mortality when compared to animals fed a standard diet [6]. All of these studies have relied on levels of serum creatinine or urea to assess renal function. It is known that both of these measurements are influenced significantly by a variety of nonrenal factors and do not provide any insight as to changes in renal blood flow. None of the above studies reported an effect of changes in lipid composition of the diet on the levels of blood pressure of animals with renal disease.

The present study examines renal hemodynamic and functional changes occurring after subtotal renal ablation and the effect of modifying the dietary content of linoleic acid. We utilized inulin and paraaminohippurate clearances to measure renal function along with repeated blood pressure measurements in awake animals. We also evaluated the effects of dietary content of linoleic acid on the degree of proteinuria, renal histology and urinary excretion of prostaglandins. Lipid composition of renal tissue was studied in normal rats and rats with subtotal nephrectomy fed the two different diets.

### Methods

#### *In vivo studies*

Studies were performed in adult female Sprague-Dawley rats (Holtzman Co., Madison, Wisconsin, USA) weighing 216 to 286 g. The rats were fed a standard rat chow containing 22.8% protein (Purina Lab Chow, Ralston-Purina Co., St. Louis, Missouri, USA) and allowed tap water ad libitum prior to surgery.

Five weeks before renal clearance studies were performed, 7 to 8 week old rats (initial weights comparable in both groups) weighing 160 to 200 g underwent 1-3/4 nephrectomy as previously described [7]. The day following surgery the rats were begun on chronically pair-fed, isocaloric diets which were either rich or poor in linoleic acid content, for five weeks. The nutritionally adequate diets [8] were prepared by Ralston-Purina. Each contained 20% fat (by weight), supplied in the high linoleic acid diet (HLAD) as safflower oil (72% linoleic acid) and in the low linoleic acid diet (LLAD) as beef tallow (2.6% linoleic acid). The diets were otherwise identical in protein (24% supplied as casein), fiber, mineral and vitamin content. The paired rats were fed their respective diets and were maintained in individual metabolic cages throughout. They were weighed daily and offered tap water ad libitum. Prior to surgery and at sequential two-week intervals (weeks 3 and 5 on diets) all animals had measurement of 24-hour urine protein excretion and systolic blood pressure (in unanesthetized rats) performed using the tail plethysmography method. For this procedure, rats were placed in a quiet, temperature controlled environment. A tail cuff was placed as proximal as possible and five consecutive blood pressure readings were recorded using a Model PE-300 Electro-Sphygmomanometer equipped with a sensitive piezoelectric transducer (Narco Bio-Systems, Houston, Texas, USA) connected to a Model 1241 Soltec recorder (Sun Valley, California, USA). Also, systolic blood pressure was measured weekly in four awake rats from each group as described above. The blood pressure values reported are the average of five determinations. At the end of five weeks of HLAD or LLAD administration, clearance studies were performed.

#### Clearance studies

Renal function in these rats was determined in the awake state using standard clearance techniques [7]. Following an overnight fast, rats were anesthetized with ether for insertion of cannulae into the femoral artery, tail vein and bladder. Animals were then placed in Plexiglas holders, and 1-1/2 to 2 hours was allowed for recovery from anesthesia and for urine flow to become stable. A priming dose of chemical inulin (Fisher, St. Louis, Missouri, USA) and chemical PAH (Merck, Sharp and Dohme, West Point, Pennsylvania, USA) was infused over a three minute period, followed by a sustaining infusion that contained sufficient inulin and PAH to maintain plasma levels. The sustaining solution was infused at 39  $\mu$ l/min. After an equilibration period of 60 minutes, urine and blood specimens were collected for clearance periods. Urine for all clearance periods was collected in previously weighed tubes immersed in iced water (to preserve the excreted thromboxane B<sub>2</sub>). Plasma and urine aliquots were obtained for each clearance period for determination of inulin, PAH, and blood urea nitrogen. An aliquot urine sample was frozen and stored at -70°C for determination of PGE<sub>2</sub>, TXB<sub>2</sub>, and 6-keto PGF<sub>1 $\alpha$</sub> .

#### Other measurements

Upon completion of functional studies in both groups of rats, the animals were anesthetized with ether, kidneys were excised, fixed in buffered formalin. Tissue was prepared for light microscopic examination by standard histologic techniques, paraffin sections were cut to 4  $\mu$ m thickness and stained with

periodic acid Schiff stain. Light microscopy to assess glomerular and small, renal blood vessel morphology was performed on the kidney of each rat. At least 50 consecutive cortical glomeruli were examined in each kidney. After the rats were killed, the heart was excised and weighed. Body weight was also determined and the ratio of heart weight (in milligrams) to body weight (100 g) was calculated [9].

#### *In vitro experiments*

*Effect of HLAD and LLAD on the lipid composition of renal tissue.* A separate group of normal rats or rats with subtotal renal ablation were pair-fed diets of high or low linoleic acid content for five weeks. Kidneys were removed under ether anesthesia and in the case of normal rats separated into cortex and medulla and stored at -70°C. In rats with a remnant kidney only the cortex could be separated. Lipid extraction and analysis of neutral lipids and phospholipids in cortex or medulla was performed using gas chromatography as previously reported from this laboratory [10].

#### *Clinical and radioimmunoassay determinations*

Inulin in urine and plasma was determined using the microanthrone method [11]. The PAH content of blood and urine was determined by a modification of the method of Smith et al [12]. Urine protein was quantitated using the method of Lowry et al [13]. Urine samples for determination of PGE<sub>2</sub>, thromboxane B<sub>2</sub> (TxB<sub>2</sub>), a stable metabolite of thromboxane A<sub>2</sub>, and 6-keto PGF<sub>1 $\alpha$</sub> , a stable metabolite of prostacyclin, were collected during clearance studies. <sup>3</sup>H-labeled TxB<sub>2</sub>, PGE<sub>2</sub> and 6-keto PGF<sub>1 $\alpha$</sub>  for radioimmunoassay were purchased from New England Nuclear (Boston, Massachusetts, USA). Urine samples were prepared by passage through octadecyl disposable columns (J. T. Baker Chemical Co., Phillipsburg, New Jersey, USA). Further purification was achieved by preparatory thin layer chromatography, using a solvent system consisting of ethylacetate: 110, isooctane: 50, acetic acid: 20 and water: 100. Eluates from octadecyl columns were evaporated under N<sub>2</sub>, redissolved in chloroform:methanol 2:1 and run on commercial silica gel G plates (Merck). Prostaglandin standards were run parallel to the samples and position determined by exposure to iodine (standards only). Silica gel from corresponding areas containing material from samples was scraped off and extracted with methanol. Recovery was calculated by adding small amounts (800 CPM) of <sup>3</sup>H-labeled TxB<sub>2</sub> or 6-keto PGF<sub>1 $\alpha$</sub>  to the urine sample before purification steps and counting residual radioactivity. Recovery rates ranged from 55% to 65% for both ligands. Determinations were made using a single antibody radioimmunoassay (rabbit antisera) and charcoal precipitation techniques. Anti-TxB<sub>2</sub> had 0.012% cross-reactivity with PGE<sub>2</sub>, 0.009% with 6-keto PGF<sub>1 $\alpha$</sub> , 0.075% with PGF<sub>2 $\alpha$</sub> . Anti-6-keto PGF<sub>1 $\alpha$</sub>  had 0.13% cross-reactivity with PGE<sub>2</sub>, 0.001% with TxB<sub>2</sub>, 2.4% with PGF<sub>2 $\alpha$</sub>  and 6.5% with PGF<sub>1 $\alpha$</sub> . Appropriate corrections for recovery rates were made when calculating the prostaglandin values.

#### *Calculations and statistical analysis*

Clearances were calculated using standard formulas. An unpaired *t*-test was used when comparing HLAD and LLAD

**Table 1.** Body weight, food intake, systolic blood pressure and ratio of heart weight to body weight in rats with subtotal nephrectomy fed diets with a high or low content of linoleic acid

Dietary group	Body wt grams			Food intake g/day	Syst. BP mm Hg			Ratio heart wt [g] · 10 <sup>5</sup> / body wt [g]
	before SNX	3 weeks after SNX	5 weeks after SNX		before SNX	3 weeks after SNX	5 weeks after SNX	
HL N = 16	193.1 ± 3.7	232.0 ± 5.1	250.0 <sup>b</sup> ± 4.0	10.1 ± 0.2	123.7 ± 2.9	149.19 <sup>a</sup> ± 5.13	156.0 <sup>a</sup> ± 5.6	397.2 <sup>a</sup> ± 14.6
LL N = 14	194.0 ± 3.4	224.0 ± 4.6	234.5 <sup>b</sup> ± 5.6	10.2 ± 0.2	128.3 ± 2.3	197.22 <sup>a</sup> ± 9.09	215.1 <sup>a</sup> ± 8.1	481.3 <sup>a</sup> ± 18.4

Values are expressed as means ± SEM.

Abbreviations are: Syst. BP, systolic blood pressure; SNX, subtotal nephrectomy; HL, high linoleic acid diet; LL, low linoleic acid diet.

<sup>a,b</sup> Significant difference between HL and LL groups,  $P < 0.01$  (<sup>a</sup>);  $P < 0.05$  (<sup>b</sup>)

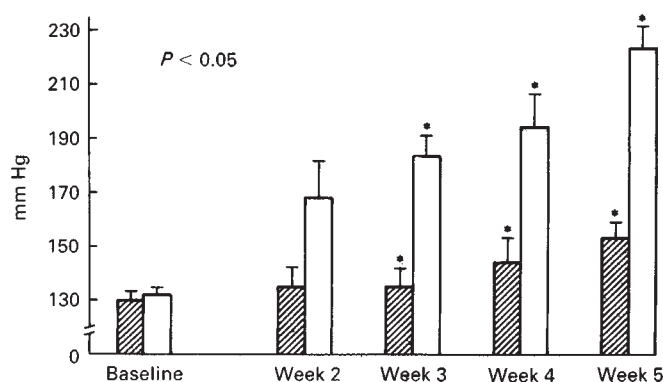
groups and paired *t*-test when comparing the data before and after subtotal renal ablation within the same dietary group.

### Results

Mortality rates were similar in both groups. Six rats out of 22 died in the HLAD group (27%). Of these, five died in the first week after surgery. In the LLAD group all five rats out of 19 died in the first week after surgery (26%). Table 1 presents data on body weight and systolic blood pressure before, three and five weeks after subtotal nephrectomy in the two groups of rats. Food intake represents average of daily food intake throughout the study. Also shown is the ratio of heart weight to body weight five weeks after subtotal nephrectomy in the two groups of rats studied. Despite comparable food intake in the two groups, the rats fed a low linoleic acid diet did not gain as much weight during this five week period as those fed a high linoleic acid diet. Systolic blood pressures before subtotal nephrectomy were comparable in both groups of rats. Hypertension became more severe in the LLAD group at three weeks. Five weeks after nephrectomy systolic blood pressure values were significantly higher in rats fed a low linoleic acid diet ( $215 \pm 8.1$  mm Hg) as compared to those fed a high linoleic acid diet ( $156 \pm 5.6$  mm Hg). The differences in systolic blood pressure between groups were also reflected in the ratio of heart weight to body weight. A lower ratio of heart weight to body weight was observed in the animals fed a high linoleic acid diet. Rats fed the low linoleic acid diet had the highest ratio of heart weight to body weight. The values were significantly different between the two groups.

Values for systolic blood pressure measured at weekly intervals in four pairs of rats fed high or low linoleic acid diets for five weeks are shown in Figure 1. Three weeks after subtotal nephrectomy systolic blood pressures were significantly higher in the animals fed low linoleic acid diets. Blood pressures continued to increase in both groups of rats but the increase was greater in the rats fed low linoleic acid diets.

Table 2 depicts mean values for inulin and PAH clearances, expressed both in absolute values and per gram kidney weight. It also presents mean values for hematocrits, weight of the remnant kidney and urinary excretion of protein in the two groups of rats. Mean inulin clearances in absolute values or per gram of kidney weight were significantly higher in the animals fed a high linoleic acid diet as compared to those fed a low linoleic acid diet. There was also a higher clearance of PAH in these animals as compared to those fed the low linoleic acid



**Fig. 1.** Values for systolic blood pressure in two groups of rats with subtotal renal ablation fed a high (stippled bars) or a low (open bars) linoleic acid diet. Three weeks and at subsequent intervals after subtotal nephrectomy there were significant differences in systolic blood pressure between the two groups. Each group was composed of 4 rats.

diet. Thus, both GFR and renal plasma flow were greater in animals fed a high linoleic acid diet when compared to those fed a low linoleic acid diet. Mean values for filtration fraction were not significantly different between the two dietary groups. Values for urine protein excretion before subtotal nephrectomy were comparable in the two groups of rats studied. However, the increase in protein excretion was significantly greater in the animals fed a low linoleic acid diet than in those fed a high linoleic acid diet (Fig. 2). Five weeks after subtotal nephrectomy protein excretion averaged 90.1 mg/24 hr in the LLAD group and 36.9 mg/24 hr in the HLAD group ( $P < 0.01$ ).

No significant differences in the urinary excretion of prostaglandins were observed between the two groups of rats when excretion was expressed in absolute terms (pg of prostaglandin per min) or per ml of GFR (pg/min/ml GFR) (Table 3).

The vascular alterations observable by light microscopy in rats with partial renal infarction have been described [14]. The glomerular involvement is focal and consists of segmental thickening of capillaries, cellular proliferation, accumulation of proteinaceous material, occlusion of capillaries, occasional adhesion of the capillary tuft to Bowman's capsule and structureless glomeruli. Focal, interstitial inflammatory cell-infiltration and dilated tubules containing hyaline casts are usually present. Morphologically, rats given HLAD had qualitatively



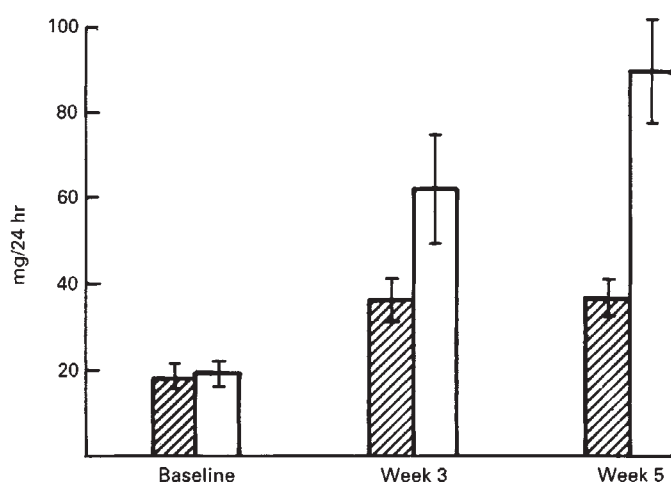
**Table 2.** Renal function, kidney weight and excretion of protein in the urine in rats with subtotal nephrectomy fed diets with a high or low content of linoleic acid

Dietary group	$C_{in}$ ml/min	$C_{PAH}$ ml/min	FF %	Remnant kidney wt mg	$C_{in}$	$C_{PAH}$	Hct %	Urine protein <sup>a</sup> mg/24 hr
					ml/min g kidney wt	ml/min g kidney wt		
HL	0.89*	2.50*	35.8	956**	1.12*	3.01*	48.29	36.9* (n = 7)
N = 16	±0.07	±0.17	±2.1	±85	±0.16	±0.38	±0.56	±4.4
LL	0.44*	1.39*	32.1	1227**	0.42*	1.26*	46.00	90.1*
N = 14	±0.05	±0.16	±1.6	±99	±0.08	±0.19	±1.62	±12.5 (n = 7)

Values are expressed as means ± SEM. Abbreviations are:  $C_{in}$ , inulin clearance;  $C_{PAH}$ , para-aminohippurate clearance; FE, filtration fraction ( $[C_{in}/C_{PAH}] \times 100$ ); HL, high linoleic acid diet; LL, low linoleic acid diet.

<sup>a</sup> Only 7 rats in each group had determination of urine protein; in the other rats food was not withheld during the collection of urine for protein determinations and it was evident on inspection that the urine was contaminated with particles of food which would have affected the measurement of protein.

<sup>b,c</sup> Significant differences between HL and LL group,  $P < 0.01$  (<sup>b</sup>),  $P < 0.05$  (<sup>c</sup>).



**Fig. 2.** Values for 24-hour urine protein excretion in two groups of rats with subtotal renal ablation fed a high (stippled bar) or a low (open bar) linoleic acid diet. Values for protein excretion rose in both groups after subtotal nephrectomy but to a lesser extent in the rats fed the high linoleic acid diet.

similar renal changes than rats given LLAD, though quantitatively the histological changes were remarkably fewer in HLAD. The incidence of normal and abnormal glomeruli in the HLAD group and the LLAD group are shown in Table 4. Using the criteria noted above, LLAD rats had  $35 \pm 5.5\%$  abnormal glomeruli whereas the HLAD group had only  $5.3 \pm 2.7\%$  ( $P < 0.001$ ).

Table 5 presents data on the effects of high and low concentrations of dietary linoleic acid on renal tissue composition in normal rats. Consistently, a low linoleic acid diet resulted in a marked decrease in 18:2 fatty acids, of both neutral lipids and phospholipids, in cortex and medulla. The concentration of arachidonic acid (20:4) was higher in animals fed a high linoleic acid diet than in those fed a low linoleic acid diet in neutral lipids of both cortex and medulla. By contrast, the content of arachidonic acid of phospholipids was not different between the two dietary groups in the cortex. There were also differences in the 18:1 fatty acid content in neutral lipids or phospholipids of both cortex and medulla. High linoleic acid diet also resulted in a significant increase of 18:2 fatty acids in the cortex of the

remnant kidney (Table 6). Arachidonic acid (20:4) content was higher in neutral lipid fractions in cortex and medulla of normal rats on high linoleic acid diet. The difference did not reach significance in rats with remnant kidneys. These data indicate that the content of polyunsaturated fatty acids in normal and remnant kidneys is altered by modifying the amount of dietary linoleic acid.

### Discussion

The present studies demonstrate that a diet with a high content of linoleic acid ameliorates the hypertension observed after subtotal nephrectomy. Differences in systolic blood pressure between rats fed a high or a low linoleic acid diet were seen as early as three weeks and the differences reached 59 mm Hg at five weeks after subtotal nephrectomy. The larger ratio of heart weight to body weight in rats fed a low linoleic acid diet indicates a greater degree of cardiac hypertrophy, presumably as a consequence of sustained and greater hypertension in this dietary group. Other factors, such as chronic anemia or hypervolemia, could affect this ratio. Similar hematocrit values in the two groups and slightly lower body weight of the rats in the LLAD group on identical food intake argue against this possibility.

The mechanisms responsible for the decrease in blood pressure in rats fed a high linoleic acid diet are not immediately apparent. Findings similar to those seen in subtotally nephrectomized rats have been reported previously in normal rats with salt-induced hypertension fed diets high or low in linoleic acid [5]. Rats fed a high linoleic acid diet had lower blood pressures than rats fed a low linoleic acid diet. However, administration of indomethacin increased blood pressure values in the rats fed a high linoleic acid diet resulting in similar blood pressures in the two groups. These data suggest a role for a metabolite of the cyclooxygenase pathway in the lower blood pressure of the rats fed a HLAD. Enhanced adrenergic activity in rats on a low linoleic acid diet was also implicated in the development of hypertension, especially in the setting of a salt load [15]. It has also been reported recently in abstract form that linoleic acid inhibits renin activity both in vivo and in vitro [16]. Such an effect may also explain the effect of linoleic acid administration on blood pressure.

Barcelli, Weiss and Pollack [3] found no differences in blood pressure in rats with subtotal nephrectomy fed high or low

Table 3. Urinary excretion of prostaglandins

Dietary group	6-Keto PGF <sub>1α</sub> pg/min	g-Keto PGF <sub>1α</sub> pg/min/ml GFR kidney wt	TxB <sub>2</sub> pg/min	TxB <sub>2</sub> pg/min/ml GFR kidney wt	PGE <sub>2</sub> pg/min	PGE <sub>2</sub> pg/min/ml GFR kidney wt
HL	324.8 ±39.71 (N = 14)	365.0 ±42.6 (N = 14)	80.69 ±22.45 (N = 8)	124.1 ±17.2 (N = 8)	306.2 ±33.66 (N = 8)	471.1 ±56.3 (N = 8)
LL	275.8 ±28.56 (N = 12) NS	452.1 ±48.6 (N = 12) NS	34.46 ±13.05 (N = 8) P = 0.05	101.2 ±11.5 (N = 8) NS	216.3 ±30.23 (N = 8) NS	480.7 ±61.4 (N = 8) NS

<sup>a</sup> Not enough urine was available for the determination of all three prostaglandins in all the rats reported in Tables I and II. Abbreviations are: HL, high linoleic acid diet; LL, low linoleic acid diet; TxB<sub>2</sub>, thromboxane B<sub>2</sub>.

linoleic acid diets. A potential explanation for the differences in blood pressure between our results and those of Barcelli et al is not immediately apparent. It is possible that the addition of soybean oil to beef tallow affected the differences between the two diets in the study of Barcelli et al [3]. Another potential difference may be the degree of renal ablation which in the studies of Barcelli et al [3] may not have been sufficient to produce a severe enough lesion (1-1/2 to 1-2/3 nephrectomy). It should be pointed out, however, that during the review process of our paper, a manuscript by Izumi et al [17] appeared demonstrating that a high linoleic acid diet decreases blood pressure in rats with subtotal nephrectomy when compared to similar rats fed a low linoleic acid diet.

Values for glomerular filtration rate (GFR) and renal plasma flow (RPF), as measured by C<sub>in</sub> and C<sub>PAH</sub> were significantly higher in rats with subtotal nephrectomy fed HLAD for five weeks when compared to those fed LLAD. In experiments utilizing protocols similar to those used in this study, we found no difference in C<sub>in</sub> between normal rats fed high (N = 4) and low (N = 4) linoleic acid diets (2.97 ml/min ± 0.14 and 2.86 ± 0.11, respectively). The conflicting information from previous studies [3, 4] regarding the beneficial effect of diets with a high content of polyunsaturated fatty acids on renal function could be attributed to high individual variability of the disease process and the reliance on the measurement of serum creatinine, rather than clearance studies to evaluate renal function. The study of Hirschberg et al did not utilize pair feeding and, therefore, differences in polyunsaturated fatty acid (PUFA) content in the diet may not have been large enough to observe differences [4].

We found no significant difference in the excretion of PGE<sub>2</sub>, thromboxane B<sub>2</sub> or 6-keto PGF<sub>1</sub> in the urine between the HLAD and LLAD groups. Similar observations have been reported for other prostaglandins of the F series [5, 18]. On the other hand, differences in TxB<sub>2</sub> and PGE<sub>2</sub> excretion between HLAD and LLAD-fed animals have been reported by others [3, 5, 19].

It has also been reported that rats with subtotal renal ablation fed a diet rich in eicosapentaenoic acid (EPA) had lower PGE<sub>2</sub> excretion, lower creatinine clearance and higher mortality than rats fed a standard diet [6]. EPA competes with arachidonic acid and is converted by cyclooxygenase into prostaglandins with different structure and lesser physiologic activity than those produced from arachidonic acid. Our data show that the tissue content of PUFA, including arachidonic acid, in renal cortex and medulla of rats can be modified effectively by

Table 4. Percentage of normal and affected glomeruli 5 weeks after subtotal nephrectomy in rats fed diets with a high or a low linoleic acid content

	Normal or slight increase in mesangial deposits and hypercellularity	Focal sclerosis	Segmental sclerosis and epithelial cell blebs	Global sclerosis
HLAD N = 14	87.1 ± 3.5	7.5 ± 1.8	2.7 ± 0.9	2.6 ± 2.0
LLAD N = 14	51.4 ± 5.8 <sup>a</sup>	13.6 ± 2.9	25.7 ± 3.9 <sup>a</sup>	9.3 ± 3.3
P	0.0001	0.08	0.0001	0.09

<sup>a</sup> Values significantly different from those obtained in rats fed a high linoleic acid diet (HLAD).

Notice that 87.1% of the glomeruli were either normal or had mesangial deposits in rats fed the high linoleic acid diet. By contrast, only 51.4% of glomeruli in the group of rats fed the low linoleic acid diet were normal or had mesangial deposits. On the other hand, 35% of glomeruli had segmental or global sclerosis in rats fed a low linoleic acid diet as compared to 5.3% in those fed a high linoleic acid diet (P < 0.001).

feeding them diets with different lipid composition, a finding which supports previous observations [20, 21]. The rather small, but significant, differences in arachidonic acid content in neutral lipid fraction of kidneys in spite of large dietary differences in PUFA in our study may reflect the paradoxical conservation of tissue arachidonate with reduced PUFA intake [15]. Prostaglandin production by kidneys after stimulation with angiotensin II was markedly reduced in animals fed a low PUFA diet [15]. This phenomenon was attributed to selective depletion of arachidonate from phosphatidylinositol in spite of overall renal conservation of arachidonate in PUFA deficiency.

The present data suggest that modification of the intake of PUFA can affect both the progression of hypertension and the decline in renal function that occur in animals with subtotal renal ablation. One of the potential mechanisms responsible for this effect can be a quantitative change in production of different prostaglandins. The data on urinary prostaglandin excretion in our study do not support this postulate. On the other hand, alteration of tissue PUFA composition may influence pathophysiologic processes by affecting cellular membrane properties and function, such as membrane fluidity [20]. Different content of linoleic acid in the diet could, therefore, significantly change the hormone receptor activity in the target

**Table 5.** Influence of high and low concentrations of dietary linoleic acid on lipid composition of renal tissue in normal rats

Fatty acids	Cortex				Medulla			
	neutral lipids		phospholipids		neutral lipids		phospholipids	
	HL N = 8	LL N = 7	HL N = 8	LL N = 7	HL N = 8	LL N = 7	HL N = 8	LL N = 7
16:0	25.49 <sup>c</sup> ± 3.75	30.79 <sup>c</sup> ± 2.16	21.51 ± 0.98	21.89 ± 0.77	17.86 <sup>c</sup> ± 2.66	23.59 <sup>c</sup> ± 1.74	19.15 ± 1.20	19.20 ± 0.47
16:1	3.43 <sup>a</sup> ± 1.12	4.81 <sup>a</sup> ± 1.53	0.81 <sup>c</sup> ± 0.19	1.61 <sup>c</sup> ± 0.45	2.66 <sup>c</sup> ± 1.04	4.66 <sup>c</sup> ± 1.18	1.14 ± 0.34	1.44 ± 0.39
18:0	7.37 ± 0.91	10.10 ± 1.21	19.29 <sup>c</sup> ± 0.5	18.08 <sup>c</sup> ± 0.56	6.00 <sup>c</sup> ± 0.76	9.44 <sup>c</sup> ± 1.08	18.48 ± 0.58	17.90 ± 0.70
18:1	17.00 <sup>c</sup> ± 3.72	28.54 <sup>c</sup> ± 5.04	8.44 <sup>c</sup> ± 1.35	13.04 <sup>c</sup> ± 1.44	22.18 <sup>c</sup> ± 3.99	43.23 <sup>c</sup> ± 5.43	11.45 <sup>c</sup> ± 1.03	17.57 <sup>c</sup> ± 2.13
18:2	27.59 <sup>c</sup> ± 5.12	10.90 <sup>c</sup> ± 2.60	10.84 <sup>c</sup> ± 1.88	5.93 <sup>c</sup> ± 1.6	41.14 <sup>c</sup> ± 5.67	12.51 <sup>c</sup> ± 8.21	12.03 <sup>c</sup> ± 1.68	4.89 <sup>c</sup> ± 1.59
20:4	5.50 <sup>a</sup> ± 1.49	4.06 <sup>a</sup> ± 1.42	31.69 ± 0.89	31.39 ± 1.21	2.16 <sup>b</sup> ± 0.73	1.28 <sup>b</sup> ± 0.46	29.43 <sup>a</sup> ± 0.98	31.63 <sup>a</sup> ± 0.40

Values are expressed as means ± SE. Statistical significance was determined using unpaired Student's *t*-test comparing fatty acid concentration between the two dietary groups in each category. Abbreviations are: HL, high linoleic acid diet; LL, low linoleic acid diet.

<sup>a</sup> *P* < 0.05

<sup>b</sup> *P* < 0.01

<sup>c</sup> *P* < 0.005

**Table 6.** Fatty acid content of the cortex of the remnant kidney

Fatty acids	Neutral lipid fraction	
	High linoleic acid diet N = 6	Low linoleic acid diet N = 7
16:0	19.29 ± 1.16	19.46 ± 0.90
16:1	0.54 ± 0.055	1.41 ± 0.13
18:0	22.56 ± 1.20	23.08 ± 1.92
18:1	9.51 ± 0.43	18.08 ± 2.56
18:2	9.88 ± 0.38	3.43 ± 0.18
20:4	18.98 ± 1.14	16.30 ± 0.90

Values expressed as % of total lipids ± SEM.

tissue or organ, affecting its long-term function as has been suggested by Hoffman et al [22]. This may occur irrespective of the effects of prostaglandins. Therefore, the changes in lipid composition of the kidney observed in our study may have contributed in whole or in part to the results obtained.

Increased glomerular fibrinolytic activity has been reported recently in rats fed a high linoleic acid diet, thus offering another possible mechanism by which increased dietary PUFA may affect progression of renal disease [23]. The beneficial effects of anticoagulant therapy in the progression of renal disease in rats with remnant kidney has been reviewed recently [24].

Whether the higher levels of systemic blood pressure were responsible for the greater functional and histological damage to the kidney in rats fed a low linoleic acid diet is not clear from the present data. We have found recently [25] that administration of heparin to rats with subtotal renal ablation decreases renal damage and systemic blood pressure despite a persistent elevation of intraglomerular pressure. These findings suggest that the systemic blood pressure elevation in these rats was secondary to the renal damage. It is possible, therefore, that the higher levels of systemic blood pressure in rats fed a low linoleic acid diet is the result of the decreased renal function and

not the cause of the renal damage. Obviously, additional studies are necessary to clarify this issue.

In summary, we have found that feeding a diet rich in linoleic acid to rats with subtotal renal ablation results in reduced hypertension and greater values for GFR and RPF when compared to rats receiving a low linoleic acid diet. Pathological changes in glomeruli were less marked in rats fed a high linoleic acid diet. The excretion of protein in the urine was also markedly less in the rats fed the high linoleic acid diet. Thus, a diet rich in linoleic acid ameliorates the progression of renal disease in rats with subtotal nephrectomy. Alteration of dietary content of linoleic acid significantly modified the lipid composition of renal cortex and medulla, resulting in higher linoleic acid and arachidonic acid content.

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